A larger material bearing on this subject could readily be gathered by the members of this Society without entailing any great trouble, and a larger material would help to clear up this difference that I have described between the cases of old cholelithiasis and those without cholelithiasis. And, as I said at the outset, it was with the purpose of enlisting your assistance that I read this paper.

In closing I may sum up the results that I have obtained as follows:

1. *Naunyn's factors—stagnation of bile plus inflammation of the bile-passage mucosa—do not seem to be sufficient by themselves to lead to gallstone formation, even though the time allowed for the working of the causes be adequate.*
2. *My first series of cases shows that these two factors lead to stone formation in patients who previously had gallstones. In this series we have the first real evidence of the factors underlying gallstone production and the causes of cholelithiasis in human beings.*

A CONTRIBUTION TO THE DOCTRINE OF THE MORPHOLOGY OF THE BLOOD, DERIVED FROM EXPERIMENTAL TOLUYLENDIAMINE POISONING.[1]

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The advances made in staining methods in recent years have increased the range and certainty of our classification of changes in the morphological elements of the blood. This was notably the case after the application of Ehrlich's method, a fact which may have led that author to state so optimistically that the doctrine of the changes in the blood, in as far as it depends upon morphological investigations, especially with the aid of color analysis, belongs to the, for the greater part, enlightened chapter of general pathology, and appears to have reached, provisionally, a definite conclusion, so that, essentially, only a few points remain to be cleared up.

Against this encouraging view is the constantly increasing list of contemporary works dealing with the blood morphology from clinical, pathological, and anatomical standpoints. As example, one needs only to mention the diversity of opinion still held as to the genesis of erythrocytes and leukocytes. Furthermore, the terms "immunity, hæmolysin," etc., of rather recent coinage, suggest possibilities of undiscovered facts just over the borderland of morphology and intimately related to the latter. It seems difficult, therefore, to exclude the possibility of morphological conditions and changes, too fine to be detected by our present technique, occurring in the blood under the influence of toxic substances, bacterial poison, etc.[1] In conclusion, it is to be noted that even the firmly established doctrine of leukæmia and "typical anæmia," as based upon clinical evidence, is constantly meeting with opposition, in the light of more recent investigations.

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[1] Based upon a series of experiments conducted under Dr. Julius Arnold at the Pathological Laboratory of Heidelberg in 1901.

Schluss-betrachtung zu der Leukaemia, Bd. viii. der spec. Pathologie u. Therapie, Wien, 1901.

In undertaking the following experimental study with toluylendiamine, we were impelled by the belief that blood morphology is still an unexhausted field containing details important for our knowledge of clinical phenomena. The mode of attack was influenced strongly by the strikingly analogous phenomena in the physiological process of coagulation as compared to the changes occurring in the blood in various anæmic conditions. It was Alexander Schmidt who pointed out this analogy.

In studying the process of coagulation, he produced artificial anæmiæ experimentally and compiled facts of intense interest and importance. To follow with understanding the points to be discussed, presupposes an accurate knowledge of the morphological processes in coagulation.[2] The necessity of being familiar with the physiological changes in the blood in judging of pathological processes is self-evident.

By employing toluylendiamine, the authors hoped to produce an artificial anæmia that might bring light to bear upon some questions of urgent clinical importance; also to have an opportunity to study such an anæmia throughout its course. In many cases of anæmia there are morphological changes to be indicated in two directions: of degeneration, of regeneration. As is well known, the differentiation is often very difficult, in fact, thus far well-nigh impossible—*e. g.*, the nature of the basophile granules in the red cells. The analogy presented by the phenomena of coagulation is encouraging, however.

Some authors maintained that many poisons produce typical morphological changes.[3] Comparing the result of our experiments with those of other investigators and inducing here the earlier results attained in studying coagulation, a further explanation of the conclusions to which we were lead seems possible.[1]

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[1] For example, the ring-like structures recently found by Cabot (Journal of Medical Research, February, 1903) in the red cells with Wright's modification of Leischmann's stain.

[2] Schwalbe. Ueber Blutgerinnung, 1900.

[3] This view was held by Heinz, Limbeck (Wiener klin. Wochenschrift, 1893), and Parlier, and opposed by Maragliano and Castellino (Zeitschrift f. klin. Medizin., Bd. xxi.). Ehrlich also expressed himself against Heinz (Ueber schwere anaemische Zustände, Verhandlungen der XI. Congress f. innere Medizin., 1892).

Through the agency of toluylendiamine, we hoped to bring new material to the question of the province of the blood plates; to get closer to the structure of the red cells, and to ascertain, to a degree, at least, the significance of the hæmoglobinæmic "inner bodies;" and finally, to verify or refute conclusions derived from earlier investigations.

Toluylendiamine[2] was first employed by Stadelmann to study icterus. His first conviction that the blood was not affected by the poison was later relinquished after Afanassiew's demonstration of the marked changes in the red cells. The drug was then used by many as a poison and Afanassiew's observations were confirmed. The action of the toluylendiamine varies greatly in different animals. As a rule, canines form the best subjects for study; moderate dosage is followed by marked icterus and high-grade changes in the blood. Hæmoglobinuria is the exception, in distinct contrast to the effect in cats, in which hæmoglobinuria is intense and almost constant, the icterus being secondary and often absent. In dogs, if the dosage is given carefully as to amount and time interval, the icterus soon disappears and the animal becomes active. In rabbits prolonged dosage can be effected without the appearance of icterus or hæmoglobinuria, but often with the production of a typical picture of severe anæmia. Rabbits, furthermore, tolerate enormous doses, especially by carefully graded increase. Guinea-pigs react much the same as rabbits; at least we have never observed icterus in either. In dogs a picture similar to that observed in rabbits can be obtained by working up gradually from very small incipient doses—namely, icterus is absent, but a severe anæmia develops. Icterus would, therefore, seem to be produced only by large single doses.

The effects of acute, subacute, and chronic poisoning were studied by means of fresh and dried blood specimens, treated by numerous staining methods. As material for investigation, chiefly dogs were used (six dogs, four rabbits, and two guinea-pigs). As to methods, there seems to be no question that wrong or one-sided conclusions can be avoided only by employing many methods and carefully comparing results. For fresh specimens, Arnold's Holland plättschenmethod proved very satisfactory. Bettmann's method of using neutral red gave interesting results. To fresh specimens, many coloring agents were applied, neutral red and methyl violet being most satisfactory. In using the latter, however, the Holland disks have to be discarded on account of the attraction of the walls of the meshes for the coloring matter. To obviate this, one can employ Neumann's method, which consists in spreading the mixture of blood and coloring agent between a cover-glass and smaller square portion of a cover-glass and inverting the former upon a smeared hollow-ground slide. The dry specimens were fixed in the usual manner; heat, alcohol, ether, formaldehyde, formaldehyde vapor, etc., are all equally reliable when properly employed. At first numerous staining methods were tried, but were finally abandoned for Laurent's solution of eosin methylene blue, Engel's method (*Zentralblatt*, 1900), and hæmatoxylin and eosin. With Ehrlich's triacid stain the results were not satisfactory.

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[1] Since the compilation of this article the views of many investigators have changed, and bear out our previous views against the specific action of various blood poisons. Bloch (Deut. Archiv f. klin. Medizin., 1903, Bd. vii. p. 277, cited by S. Batty Shaw, Practitioner, vol. lxxiii. No. 4), for example, in studying pernicious anæmia concludes that the same "must be due to some agent which interferes with the activity of the blood-forming organs," and found no evidence to support any toxic nature of this agent, "whether arising from disturbed internal secretory activity of any organ, or from digestive disturbance due to atrophy of the mucosa of the bowels, or to the occurrence of bacterial invasion." He also found that ingestion of toluylendiamine produced nothing more than secondary anæmia.

[2] Discovered by Hoffmann in 1861 as a secondary product in making aniline dyes.

A detailed report of the records seems superfluous and liable to prove monotonous, being prolonged in some cases over months. Also a tabulated comparison of blood examinations, as first planned, would add nothing in clearness. The essential is a classification of the morphological changes observed and a description of the individual groups with illustrations. The literature of toluylendiamine poisoning can be dispensed with here for brevity's sake, careful study and review of the same being understood; also, all histological and chemical changes, such as were discussed in considering the processes of coagulation. That any such study as the present requires careful macroscopic and microscopic examination of all the organs as well, is self-evident. In the following we are concerned, however, only with a description of the blood as studied morphologically in fresh and dried specimens:

I. Changes in the red cells.
  Blood counts in anæmic conditions. Degeneration. Regeneration.
  a. Phenomena of budding. Process of expulsion of parts of red cells. Question of the blood plates.[1] Intracellular hæmoglobinæmic bodies.
  b. Other phenomena of degeneration.
    Poikilocytosis. Polychromatophilia. Microcytosis. Macrocytosis. Shadows.
  c. Granules in the red cells.
  d. Appearance of nucleated red cells in the blood and loss of nucleus in same.

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[1] The classification of blood plates under red cells, and not separately, is not in accord with many recent articles on the subject, but is the result of conclusions stated elsewhere (Schwalbe, Anatomische Anzeiger, 1900, Bd. xx.), for it is not possible to regard the blood plates as other than products of the blood cells, especially of the red cells.

II. Changes in leukocytes.

Results of counts. Granules in white cells. Changes in the white cells not being recognized as referable especially to the action of toluylendiamine will be treated secondarily.

The various investigators are agreed, as a glance at the literature will show, that more importance is to be attached to the changes in the red cells than to those in the leukocytes. That the red cells are markedly influenced by toluylendiamine is clear from the clinical course of the chronic poisoning, in which all the features of a high-grade anæmia are present, intense emaciation and striking pallor of all visible mucous membranes. The blood count as obtained by Biondi and Bernhardt, also by Heinz, confirms the clinical picture. The blood counts made during the present study correspond in every case, the unusual oligocythæmia being readily recognized. One source of error should be considered, however; as mentioned in the literature, the red cells of poisoned subjects have a greater tendency to disintegrate, that is, a diminished resistance. It is certain that the conditions under which cells are counted further diminish their resistance, as a result of which the count obtained may be lower than is the case. Even with this factor considered, the oligocythæmia is sufficiently positive to be notable. By means of the count the possibility is excluded of the strikingly pale blood being caused somehow solely by decrease in the quantity of hæmoglobin.

One of the most interesting phenomena observed in the red cells is connected with the formation of the blood plates, the budding of the erythrocytes. The process is studied most satisfactorily in fresh blood specimens from a dog freshly jaundiced by toluylendiamine when examined in the Holland disks as described above. The common "thorn-apple" forms are seen in great numbers. Presently the budding process begins, the buds varying in size. A protrusion forms upon some part of the red cell, the part affected often showing a lustre differing from the rest of the cell. The projecting part becomes hemispherical, and, often with amœboid movements, gradually becomes more detached from the cell till the uniting pedicle divides the "pre-existent" blood plate.[1] Two points worthy of note are that from the outset of the study the number of blood plates and amount of budding were greatly in advance of the same observed in normal blood, and that an extensive destruction of red cells took place in fresh blood while under observation. The inference is that the same sort of disintegration takes place as in coagulation, only much more rapidly under pathological conditions. In other words, the resistance of the red cells is greatly lessened. An observation should be noted, which, without leading to definite conclusions, presents the possibility of significant deductions for the morphology of coagulation, namely, that in a dog in a condition of chronic poisoning at a time when, without icterus, a marked anæmia was present, surprisingly few changes were found in the blood in fresh specimens. This suggests invitingly the conception that in such cases the less resistant red cells succumb, the more resistant alone being left in the circulation. Its ultimate connection with the process of coagulation can merely be indicated. It is known that in artificial anæmias—*e. g.*, by repeated venesection, the coagulability of the blood at first increases, then gradually diminishes; also, that this decrease can reach a serious point. No attempt was made, in fact, no method is extant, to determine the rate of coagulation, so that any conclusions are impossible. These facts are of apparent interest, however, in assuming the possibility of some connection between these morphological changes and the process of coagulation.

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[1] It is to be clearly understood that the movement of the bud in this process is not to be confused with the Brownian movement of particles, or attributed to capillary attraction or currents in the serum. The recent studies of surface tension by J. Traube (Pflüger's Archiv, December 1, 1904) bid fair to ultimately explain many, if not all, of the phenomena of amœboid movements and disintegration (budding of the blood plates, etc.) of the morphological elements of the blood.

In toluylendiamine poisoning the budding process is, therefore, apparently greatly increased, although this cannot be stated as a fact from simple observation of fresh specimens, especially when it is known that the budding process occurs with some irregularity in coagulation and that in toluylendiamine poisoning no method is known by which to accurately determine these changes. By admixture of some coloring fluid—*e. g.*, methyl violet salt solution, as described elsewhere,[1] as emphasized recently by Bloch and Heinz, the changes become more significant. Applied to normal blood, no coloring is visible at first in the red cells. As the budding process begins, many of the buds become more or less deeply colored with methyl violet. The red cells send out plump projections, staining blue in the same manner that the "pre-existent" blood plates stain with this fluid. After prolonged observation many red cells appear to contain a granule stained blue more or less deeply, as described elsewhere.[2] These blue granules seen in the red cells of normal blood resemble entirely the blue granules described by Heinz. In the blood of animals poisoned with toluylendiamine, the process is quite different. The blood from a dog jaundiced with toluylendiamine presents a beautiful picture; immediately after withdrawal the red cells are almost uniformly occupied by blue granules and blue projections, the greater number having only one visible blue granule; some having several. On careful inspection it is evident that all of the blue particles are projections; that they are the same as those seen in studying coagulation. Fig. 1, *a* to *e*, shows several of the forms seen immediately after withdrawal; many red cells have retained their form; some contain unstained as well as stained projections. All, or nearly all, of the red cells present this picture in the blood of jaundiced dogs; in rabbits or dogs which, though repeatedly poisoned, remain unjaundiced, the number is fewer. These phenomena are interesting in several respects: First, they challenge to a comparison with other poisons; Heinz, who has determined the effect of many poisons, found the same manifestations produced by some of the poisons when studied with gentian violet salt solution. He called the stained particles in the red cells "blue granules." Bloch regarded these blue granules as products characteristic of pyridin poisoning, and affirms that he did not find them in cases poisoned with toluylendiamine. That the "blue granules" of Heinz and blue bodies (Körperschen) of Bloch are identical with those found by us can be derived from the drawings of these two authors. This would seem to indicate that these phenomena are not characteristic for any particular poisons,[1] a point made more clear by comparison with the processes observed in coagulation, in which, in normal blood, the blue granules appear in the same manner, but in fewer numbers, and only after coagulation has commenced, whereas, in poisoned blood the granules are seen immediately on withdrawal. In both instances, therefore, we have apparently to deal with an injury of the erythrocytes, a degeneration, the difference in the blue granules and projections being quantitative rather than qualitative, the degenerative process in cases of poisoning occurring after the type witnessed in coagulation. It is possible, of course, that all poisons do not act upon the red cells in the same way, but produce types of degeneration too subtle for the present technique; the rapidity and energy of the injury to the blood cells is apparently of the same importance for the production of the morphological picture of poisoning, however, as the nature of the poison.[2]

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[1] Schwalbe. Münch. med. Wochenschrift, 1901.

[2] Schwalbe. Anatom. Anzeiger, Bd.xx.

Some few poisons act by choice upon the erythrocytes; others cause the production of methæmoglobin. The distinction is evident. In staining with methyl violet salt solution possibly the blue granules predominate in some forms of poisonings, whereas, in others (still beyond our power of detection) they might be much less numerous and conspicuous. If, however, there were changes in the red cells in a sense characteristic of certain poisons, or which only appear under the action of a particular blood poison or chemically allied substances, no evidence of such was found in these investigations, or is presented in the literature (Ehrlich, Limbeck, Maragliano, Castellino, and others). It seems further impossible, in view of the fact, as stated, that all degenerative processes in the red cells, so far as it is possible to determine them with or without the aid of staining fluids, find their prototype in the process of coagulation. It remains to explain the relationship of the phenomena witnessed in studying coagulation and those obtained more recently, by means of methyl violet salt solution, to the "inner bodies" of Ehrlich (Hæmoglobinaemische Innenkörpenschen).

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[1] In Heinz's more recent publications, the conception of specific changes for certain poisons is emphasized less strongly than in his earlier works.

[2] Ehrlich said in regard to the changes described by Heinz: "Personally I am of the opinion that in higher animals this degeneration will be produced by the majority, perhaps by all, of the blood cell poisons." (Verhandlungen d. XI. Congr. f. innere Med., p. 40.)

In fresh specimens the red cells are often filled with granules which have a stronger lustre than the rest of the cell. On closer inspection these are seen to be projections which are markedly hæmoglobiniferous. An unequal distribution of hæmoglobin in the structure of the erythrocytes in the most diverse ways is met with in fresh as well as dried specimens. Ehrlich regarded the hæmoglobiniferous "inner bodies" as the result of a degeneration of hæmoglobiniferous parts of the cell. In dried specimens the projections, which in the fresh specimens stain deeply with methyl violet, are eosinophilic, as direct comparison teaches. In fresh and dried specimens made simultaneously from an animal poisoned with toluylendiamine, the former show beautiful blue projections uniformly throughout the field, the latter (stained with eosin-methylin blue or hæmatoxylin) only occasional, faintly blue-tinged projection, among the red cells. In the one case they are, therefore, strongly basophilic; in the other eosinophilic, and occasionally also faintly basophilic. These qualities, taken into consideration with the altered appearance of the projections in unstained fresh specimens, point strongly to degeneration of the hæmoglobiniferous part of the cells. This is Ehrlich's view also, for in his publications he affirms the identity of the granules described by Heinz in his study of poisonings, with the "inner bodies." Conditions closely related to, if not identical with the "inner bodies," are present without question in toluylendiamine poisoning, and the transition to blood-plate production is a slight one, as will be explained. In dry specimens the projections are often beautifully shown, and of various sizes. Naturally conservatism is necessary in regard to the value of the appearances in dry specimens, as similar changes are seen as artefacts in the process of preparation. When, however, such buds are seldom seen in the preparation of hundreds of dry specimens from normal blood, in contrast to their almost constant presence, uniformly scattered throughout the field, in the blood from toluylendiamine subjects; when, furthermore, one can demonstrate the agreement of fresh and dry specimens, it would seem permissible to utilize dry specimens in the analysis of the findings.

Blood plates occur in dry specimens in clusters in the well-known manner; also separately, often in surprising numbers. Also the forms known as blood disks, with and without the presence of hæmoglobin. Stained with methylene blue or hæmatoxylin and eosin, the blood plates sometimes show a slight metachromosis, a tone partaking of the blue of the basic stain and of the pink of eosin. A "nuclear" structure is often apparent, stained exquisitely blue, as described earlier by Arnold, and as emphasized recently by Deetjen especially (who, to be sure, gives another significance to it). Some of these plates, mostly of a delicate form, appear as if caught in amœboid movement in the preparation of the specimen, discrete or attached to a red cell, and with the nuclear structure stained blue (Fig. 2). That they are identical with Deetjen's findings is evident from a comparison of the drawings.[1]

Since Hayem and Bizzozero called attention to the blood plates in the seventies (they had been noted casually before by many authors) these bodies have claimed greater interest. The two essential questions in regard to them, their origin—thereby the question of pre-existence—and their significance in the processes of degeneration and coagulation, are still unsettled. Three views are extant as to their genesis; that they are thrombocytes (Dekhuysen), that is, independent pre-existent bodies in the blood, having the physiological importance of separate structures and constituting an essential third element of the blood, requiring explanation as to development and origin; that they are not pre-existent, but merely artefacts, globulin sediment and products of precipitation—an opinion with few retainers at present; that they are derivatives of the blood cells, either of the red cells, or white cells, or both. For a long time the leukocytes were regarded as the sole source by many eminent investigators. An extensive list of publications show, however, that the red cells are regarded as sharing at least in their production.[2] The participation of the blood plates in the process of coagulation has been observed repeatedly since Hayem and Bizzozero, and its significance variously interpreted. The works of Arnold, Franz Müller, Feldbausch, and Schwalbe should be mentioned in this connection. From prolonged observation of the budding of the erythrocytes in fresh specimens, with and without the admixture of methyl violet salt solution, it is difficult not to connect this phenomena with the increased production of blood plates. This was also the observation made by Arnold, and later by Schwalbe, in studying coagulation, and which led to the same conclusion, namely, that the blood plates are buds from the erythrocytes. As previously stated, blood plates were found in large numbers in fresh and dry specimens from poisoned dogs. In dry specimens the blood plates often found attached to the erythrocytes can be explained in two ways: either that a bud has been caught in the act of emerging from the red cell—a process often seen in fresh specimens—or that a blood plate has wandered to the red cell and become adherent—a process that unquestionably also occurs frequently. But to determine in the dry specimen between the two is hardly possible. One feature is striking, however: in Fig. 2 red cells are seen with a dell at the point of attachment of the blood plate, giving the impression of a loss of substance at that point. In such cases it may not be too daring to assume that the loss of substance is due to exodus of the blood plate at that point. Furthermore, comparing the fresh and dry specimens: in the dry specimens the supposedly budding blood plate contains a nuclear-like structure stained distinctly with hæmatoxylin, the "nucleoid" of Arnold, the nucleus of Deetjen, Dekhuysen and Kopsch.

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[1] Virchow's Archiv, Bd. clxiv.

[2] Interesting in this connection is Stengel's excellent review of the recent literature (Progressive Medicine, June, 1902), and his views in support of the latter theory, published after the initial compilation of this paper.

Fig. 2, *h*, shows a blood plate with nucleoid, and in addition, hæmoglobin stained with eosin. This observation, namely, of blood plates that contain a nucleoid body staining with hæmatoxylin or methylene blue, and that are also hæmoglobiniferous and stainable with eosin, has been seen repeatedly, not only in the pathological specimens, but also in normal blood. Unless this be purely adventitious, it speaks clearly for the erythrocytic origin of the blood plates. It should be emphasized again that the findings are taken into account only in support of this view and that the comparison of fresh and dry specimens is very striking. The conviction that the blood plates are divided from the blood cells is founded not only upon the phenomena studied in fresh and dry specimens under normal and pathological conditions and upon the observations made in following the process of coagulation; it is also verified by Arnold's investigations of circulating blood, especially his extensive study of thrombi treated by the most diverse methods. The objections to the deductions of Deetjen and Kopsch and the opinions of Hirschwald have already been published.[1]

The usual changes present in anæmic conditions were also found in toluylendiamine poisoning, namely, the so-called shadows (to which Afanassiew referred especially in connection with toluylendiamine poisoning), poikilocytosis, and polychromatophilia. In how far the latter is to be regarded as an indication of degeneration is beyond discussion here. The striking variation in the size of the red cells, especially the presence of very minute forms, is noteworthy.

As to the granules in the red cells: basophile granulation of the red cells was described at an earlier period by Askanazy and others, but not until after the studies of A. Plehn did any great number of investigators begin to turn any particular attention to them. Among the more recent work should be mentioned that of Grawitz, Litten, Hamel, Bloch, Isserlin, Cabot, Fisch, and Stengel. Plehn, whose views are compiled and beautifully illustrated in the monograph of 1901, believes that certain basophile granules of the erythrocytes, found in the blood of malarial patients subsequent to recovery, are to be regarded as latent forms of the plasmodium. It is interesting that later, after Plehn's view was rather generally refuted, Bloch, after examining Plehn's specimens—having previously disputed Plehn's contention—came to the conclusion that the granules in Plehn's specimens were with great probability of a different nature from those described by himself and others, and that therefore Plehn's view could not be rejected without further proof. If Plehn's view is correct, it presupposes the existence of two distinct kinds of basophile granules; one characteristic of latent malarial parasites alone; the other common to, if not constant with, the most diverse forms of anæmia; the anæmia of Biermer, of carcinoma, of different toxic conditions. It is difficult to reconcile the present literature with Plehn's view, though only a systematic study of malarial blood would justify any conclusions.[1]

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[1] Schwalbe. Anat. Anzeiger, Bd. xx.

The granules seen in toluylendiamine poisoning correspond throughout with those described by Grawitz, Litten, and others. In dog's blood they are not constantly present. Guinea-pig's blood gives the most beautiful granules, both under normal and pathological conditions. In normal blood of healthy rabbits the granules are also present, but less frequently than in guinea-pigs. Bloch states that he did not find them in normal blood of guinea-pigs. Engel found them in the red cells of embryonal mice. In the guinea-pigs studied there was a marked variation in the size of the granules, both in the polychromatophilic and otherwise normal red cells. In dog's blood, in which, as stated, they were present only in poisoned cases, the variation in size was even more marked. They were most frequent in the chronic cases, rare in acute cases. The large granules are to be regarded apparently as "nucleoid" (Fig. 3, *c-e*). These resemble the nucleus of the blood plates described by Deetjen. In Fig. 3, *a*, *b*, *f*, and *k*, the granules or nucleoid is surrounded by an apparent concentration of hæmoglobin, that is, a "hæmoglobinæmic inner body," with nuclear substance. Prolonged observation of such forms raises the question as to whether they may be preparatory forms of blood plates in the stage previous to expulsion; at any rate, the indication is not easily disregarded.

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[1] Since this paper was first drafted an opportunity was offered in June and July, 1902, to study a series of over thirty cases of malaria in New York. They were all tertian, six were double. In only two cases were granulations present in addition to a frequent poikilocytosis and polychromatophilia. In all others no granulations were found even weeks after recovery. Jenner's and Laurent's stains were used, the latter bringing out the variations in color in the manner produced by Wright's modification of Leishmann's stain. The two cases presenting granules in the red cells were in adults with a double tertian infection of three and four weeks duration respectively. In the specimens from both cases numerous granulated red cells were found containing in addition young hyaline bodies (Figs. 25, 26). The granules corresponded throughout with those seen in toluylendiamine poisoning, in some instances being large and fewer to a cell, in others smaller and more evenly distributed, especially throughout the periphery, with a tendency to ring formation. One of these cases presented normoblasts, and among them cells like those reproduced in Fig. 3, *i*, and Fig. 4. In neither case could hyaline bodies be found resembling in any way the large granules in Fig. 4; and, vice versa, one could not easily mistake such large granules for even the youngest hyaline bodies. These large granules correspond in size, form, and staining qualities, however, to the granules described and reproduced by Plehn as latent malarial organisms, and were found repeatedly, as mentioned, in the blood of toluylendiamine subjects. In the two cases cited, the large granules disappeared after administration of quinine, just as in the dog after cessation of the toluylendiamine dosage.

As to the smaller granules, two views exist as to their province; that they are products of nuclear degeneration of nucleated red cells; that they are the expression of degeneration of the hæmoglobiniferous protoplasm. It is not inconceivable that they are evidence of regenerative processes. Bloch, who discusses these phenomena at some length, regards them as evidence of protoplasmic degeneration. One fact against the view that they are connected with nuclear degeneration is that they are found in red cells whose nuclei are undergoing mitosis (Fig. 12). Until further evidence can be produced, however, Bloch's view must remain unchallenged.

In mitosis the entire cell is concerned, protoplasm and nucleus both being involved in the process of division. It seems inconceivable that nuclear degeneration is to be inferred from the presence of granules in a cell whose nucleus is in a condition of growth. The same argument is to be brought with scarcely less probability against protoplasmic degeneration. Assuming that the protoplasm and nucleus of a dividing cell are under the same conditions, the protoplasm must also be in a state of productive activity. The view is not untenable, however, that the nucleus, in preparation for mitosis, rids itself of certain useless material which is manifested in the shape of granules. The same may be true of the protoplasm. Bloch's findings present nothing definite for or against nuclear or protoplasmic degeneration or even the possibility of some other significance. The deductions of Engel, establishing these granules as nuclear fragments, are at least not disproved by Bloch.[1]

In studying the process of coagulation in normal blood, basophile granules were found, which stained with methyl violet and varied in size like those seen in fresh specimens in toluylendiamine poisoning, the latter being mostly large and regarded as being associated in some way with the production of blood plates. The finer granules found in dry specimens cannot be classified unqualifiedly with the above. Whether they are similar to the finer granules seen in fresh specimens when stained with other fluids—*e. g.*, neutral red, is an open question beyond discussion here.

As to the granules being nuclear fragments or products of degeneration of the hæmoglobiniferous protoplasm or capable of other interpretation, a uniform explanation, in the sense that only one or the other possibility is to be admitted, seems improbable. Both may apply. Still another possibility is that cited by many investigators, especially Arnold, namely, that the protoplasm of the red cells may have a granular structure, hidden by the hæmoglobin under ordinary circumstances. Clinical experience speaks fully for the existence of changes in the hæmoglobin leading to destruction of the same without disintegration of the cell form, as, for example, in the so-called shadows. One is often reminded of this possibility by appearances observed in the red rells in fresh and dry specimens, and of the further possibility that such conditions may be revealed to us by the aid of finer technique.

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[1] Cabot (Journal of Medical Research, February, 1903) thinks the granules found in various anæmic states may be due to over-ripeness of the cell or to degeneration of the protoplasm. But with Schmidt and others he believes that they are derived from the nucleus, and not from the protoplasm, whenever changes occur in the nucleus of a degenerative or regenerative character. The ring-like structures found by Cabot in some cases of pernicious anæmia, lead-poisoning, and lymphatic leukæmia mentioned in a previous foot-note, are regarded by that author as having some possible connection with the granules, and as probable derivatives of the nucleus in the same way as the basophile granules. Unlike the latter, however, they do not stain with hæmatoxylin. No special significance is attached to them.

In toluylendiamine poisoning, as in every case of anæmia the course of which is not ended rapidly by recovery or exitus, the process of degeneration is accompanied by an attempt at regeneration. It has been established that even after intense icterus the animal can recover. Nucleated red cells are found as an expression of this attempt at regeneration. Ehrlich classified normoblasts, megaloblasts, and microblasts. In toluylendiamine poisoning normoblasts were preponderant, megaloblasts being rare. It is well known that a differentiation depends more upon nuclear characteristics than size, and that it can at times be very difficult to distinguish between the several forms. In rare instances very minute nucleated forms have been found (Figs. 20, 21) that must be regarded as microblasts. Further description of the various forms observed is not essential here (Figs. 5 to 21). Many can be classified as normoblasts by the nuclei apart from the size. Some nuclei contain a more deeply stained nucleolus. In some instances mitosis is present (Fig. 16); in others two discrete nuclei are found in a cell and the protoplasm "laced in" between them (Fig. 18). This observation of mitosis in the circulation has been made repeatedly. Triform division of the nucleus has also been seen in the specimens.

INDEX TO ILLUSTRATIONS.

FIG. 1,[1] *a–e*. Dog V. Red blood cells as observed in fresh specimens. Methyl violet salt solution.

FIG. 2, *a–p*. Dog I. Blood plates with nucleoid, in part free, in part attached to red cells: *e*, *f*, *g*, show cell at point of attachment; *h*, red blood cell with blood plate containing nucleoid and hæmoglobin stained with eosin. Hæmatoxylin and eosin.

FIG. 3, *a–k*. Different-sized granules in the red cells, hæmatoxylin and eosin: *c*, the large blue-stained granules represent the "nucleoid" of the blood plate, also in *d* and *e*; *i*, nuclear degeneration and fine granules; *j* and *k*, fine granules and a large granule.

FIGS. 4–8, 10–12. Dog VI. Nucleated red cells with granules. Laurent stain.

FIG. 8. Polychromatophilic nucleated red cell with granule.

FIG. 9. Guinea-pig. Nucleated red cell with granules. Laurent.

FIG. 13, *a*, *b*. Dog II. Nucleated red cells. Hæmatoxylin and eosin.

FIG. 14, 15, 16. Dog I. Nucleated red cells. Hæmatoxylin and eosin.

FIG. 22. Expulsion of nucleus.

FIG. 23, *a*, *b*, *c*. Dog IV. Small lymphocytes. Eosin methylene blue.

FIG. 24. Normal red cell for gross comparison.

FIGS. 25, 26. Red cells containing granules and hyaline malarial organisms.

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[1] Fig. 1 drawn by Schwalbe, the rest by Solley.

FIG. 1

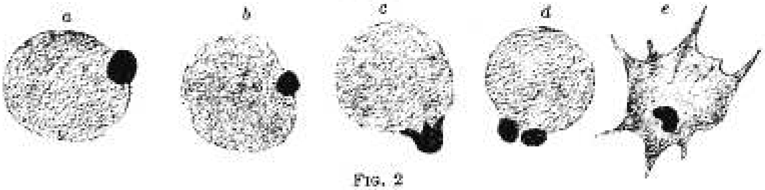


FIG. 2

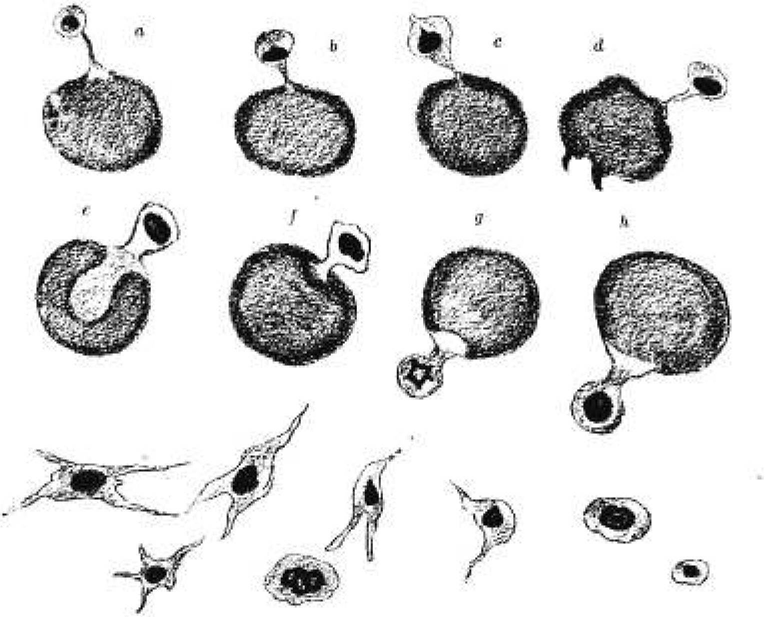


FIG. 3

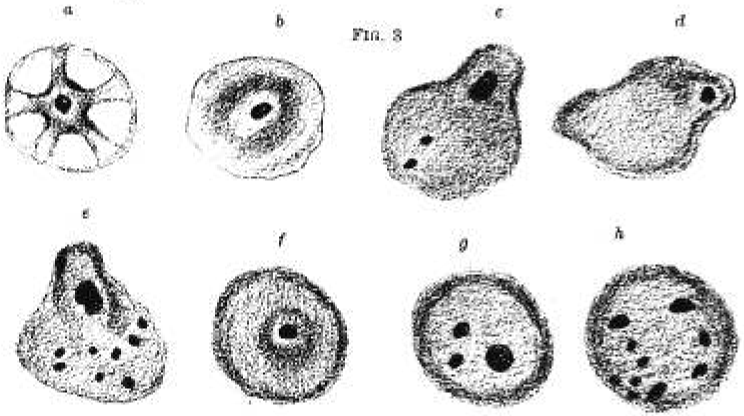


FIG. 3

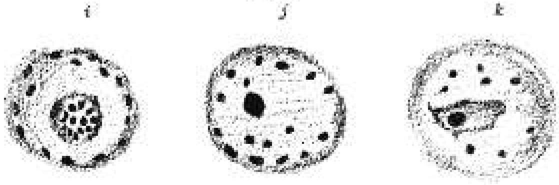


FIG. 4

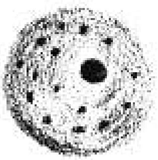


FIG. 5

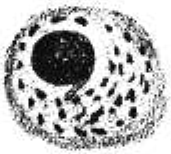


FIG. 6

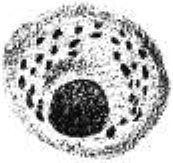


FIG. 7

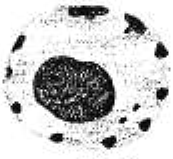


FIG. 8

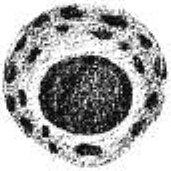


FIG. 9

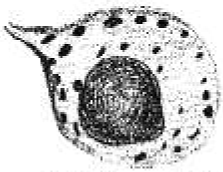


FIG. 10

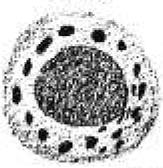


FIG. 11

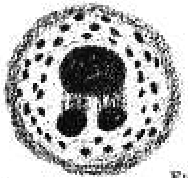


FIG. 12

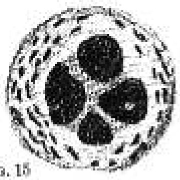


FIG. 13a

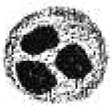


FIG. 13b

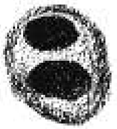


FIG. 14

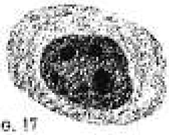


FIG. 15

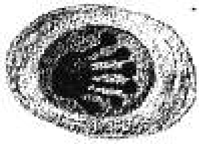


FIG. 16

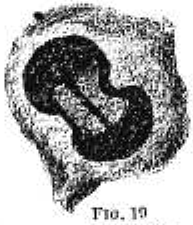


FIG. 17

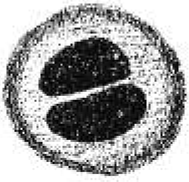


FIG. 18

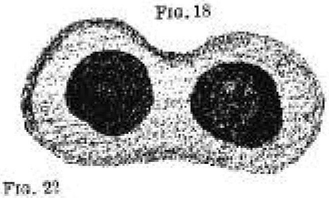


FIG. 19

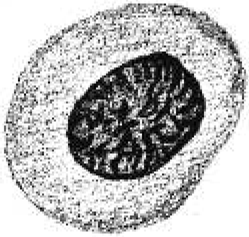


FIG. 20

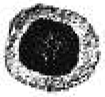


FIG. 21

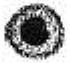


FIG. 22

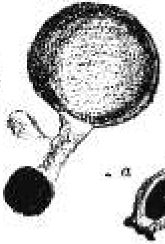


FIG. 24

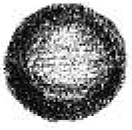


FIG. 23

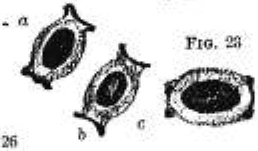


FIG. 25

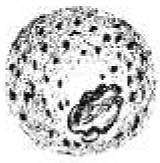
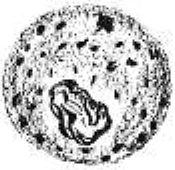


FIG. 26



The question as to how the nucleus of the red cell disappears can be little influenced by the present study. There were numerous instances of free nuclei in the blood and of others in the act of expulsion (Fig. 22). Disintegration seems to go hand in hand with expulsion, but it is impossible to determine how much of this is natural and how much artificial. The relation of the granules to nuclear degeneration has already been discussed. An instance of nuclear degeneration is shown in Fig. 3*i*, one of many found in the blood from a dog during acute poisoning. In fresh specimens the nuclei stain rapidly and beautifully with methyl violet. There is no apparent advantage in Bettmann's method of employing neutral red, applied by that author in studying the blood of embryonal mice.

That the white cells are little affected by toluylendiamine is shown by observation, as well as a study of the literature. Accurate counts usually denoted a slight leukocytosis, never a hypoleukocytosis. The increase must generally be regarded as relative, however. Biondi claims to have found a leukocytosis to twice or thrice the normal count and regarded it as absolute. In the present observations such counts were rare and cannot be regarded as absolute, in view of the impossibility of employing a counting method that will exclude errors due to disintegration of the red cells and the constantly changing proportion of morphological elements to serum under the influence of the toluylendiamine dosage. There were no positive indications at any time of degeneration of the leukocytes, although the nuclei were often strikingly pale. A peculiar form of small lymphocyte (Fig. 23, *a*, *b*, *c*), found in several cases, is worthy of mention. Interesting granules were found in the leukocytes, which could not be referred to specific action of toluylendiamine, and which could not be considered here without regard for the enormous literature, that would exceed the present purpose. Hirschfeld, in his comparative investigations, concludes that the polynuclear eosinophile leukocytes are common to all mammalia, but that every species possesses in addition polynuclear leukocytes with specific granules. The human species possesses in its neutrophile granular leukocytes a specific form found in no other animal. In poisoned rabbits, beside eosinophile and pseudoeosinophile leukocytes, certain forms were found with large red granules, which, by reason of their arrangement and unusual size, could not be classed with eosinophiles. Others were found with violet granules of varying sizes, sometimes varying in the same cell, often crowded together throughout the protoplasm. The doctrine of the granules is such a disputed one as to make impossible any attempt to discuss the findings of the present study. The staining quality of the granules alone does not apparently present a fundamental distinction, but rather stands in direct relation to the condition of the cell function. (Arnold; Hesse, Virchow's *Archiv.*, Bd. clxvii.)

In conclusion, the changes found in the pathological processes produced by toluylendiamine poisoning are the same as those observed in many other toxic and anæmic conditions and find their analogy essentially in the changes seen in the study of physiological degeneration. If the purpose of these pages is accomplished, they will serve as a help or incentive to others to use these observations and deductions in studying and attempting to shed new light upon the manifold and complex questions of blood morphology.

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XEROSTOMA.

By Lewis S. Somers, M.D.,

of Philadelphia, Pa.

While a relative degree of aptyalism, or more correctly partial suppression of the buccal and salivary secretion, is not uncommon as a result of the administration of atropine, or occurs during the course of various infections such as typhoid fever, and is also dependent upon long-continued mouth breathing from obstructing adenoid masses in the nasopharynx, yet as an independent affection *per se* this condition of xerostoma, or dry mouth, is exceedingly unusual.

Symptomatically, the affection consists essentially of a great diminution or almost complete arrest of both the secretion of the salivary glands and the numerous mucous glands of the buccal interior, while the symptom-complex thus produced evinces such a clear and definite picture that but little basis exists for confusing it with any other condition. What may be designated relative xerostoma is also found, in addition to the conditions previously mentioned, in diabetes and as a physiological state in old age to a moderate degree, while a somewhat analogous condition has been described by Heerat as psychic salivation. In four cases of the latter, which he studied, there was continual spitting without apparent cause, the patients considering it necessary to get rid of the mouth secretions. The saliva then became repugnant to such a degree that it was no longer swallowed, and as a result of this psychic state the glands were at first stimulated to increased secretion, which the patient constantly expectorated; while as a later phenomena dryness of the mouth resulted. In true xerostoma, however, while the excessive dryness is the most prominent symptom, yet other evidences of a more serious nature are associated with it and, as in the few cases recorded since it was first recognized by Hutchinson, the affection seems to deviate but little in every instance, it occupies